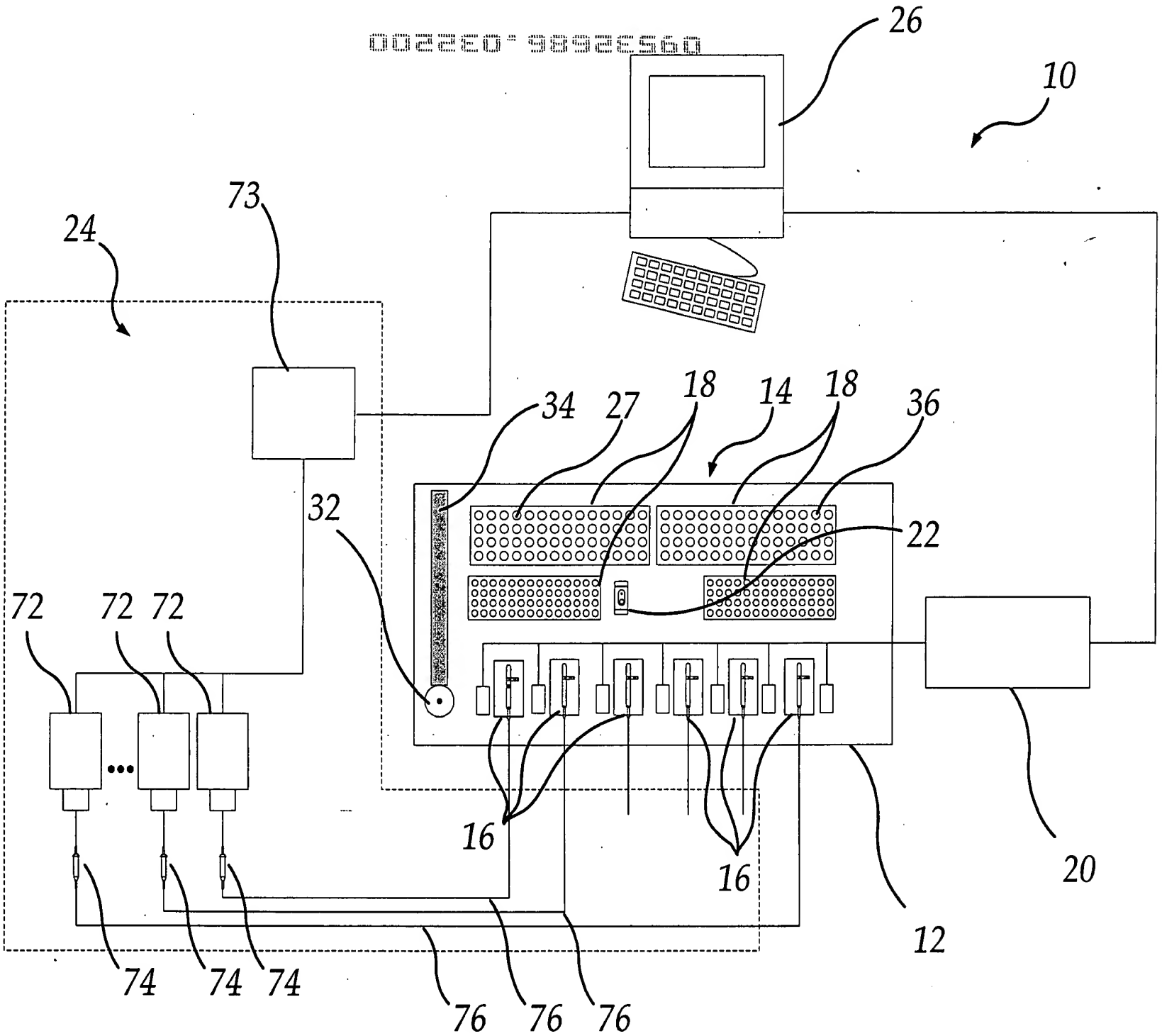

26
10
24
73
34
27
18
14
18
36
32
22
72
72
72
16
16
20
12
74
74
74
76
76
76

FIG. 1

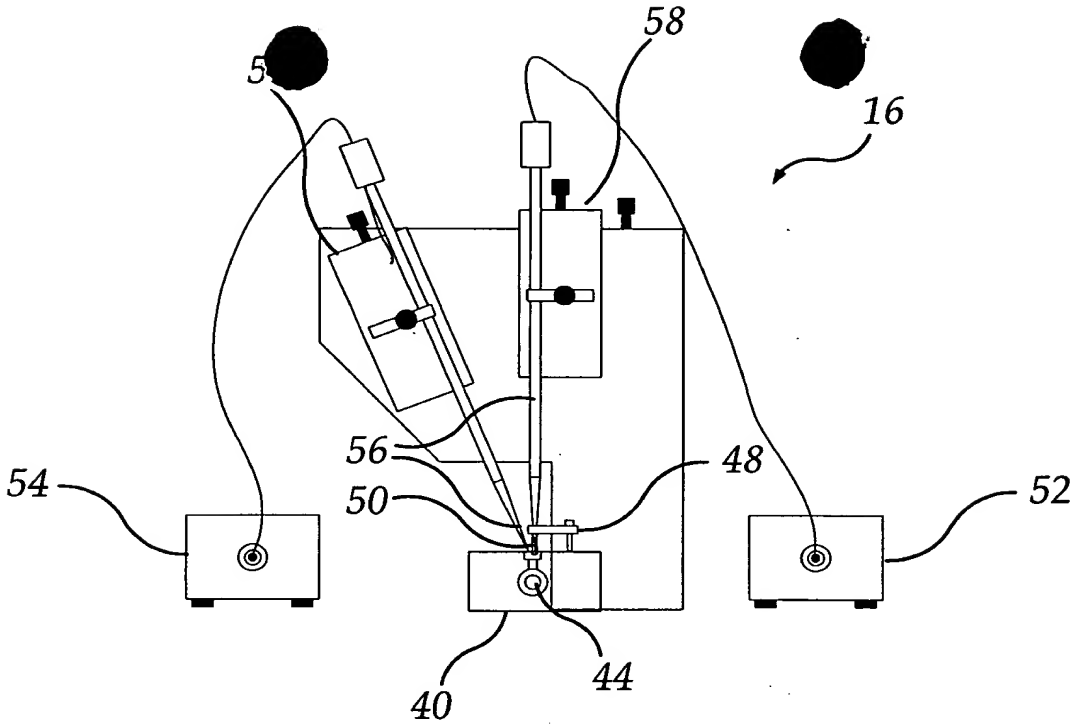


FIG. 2

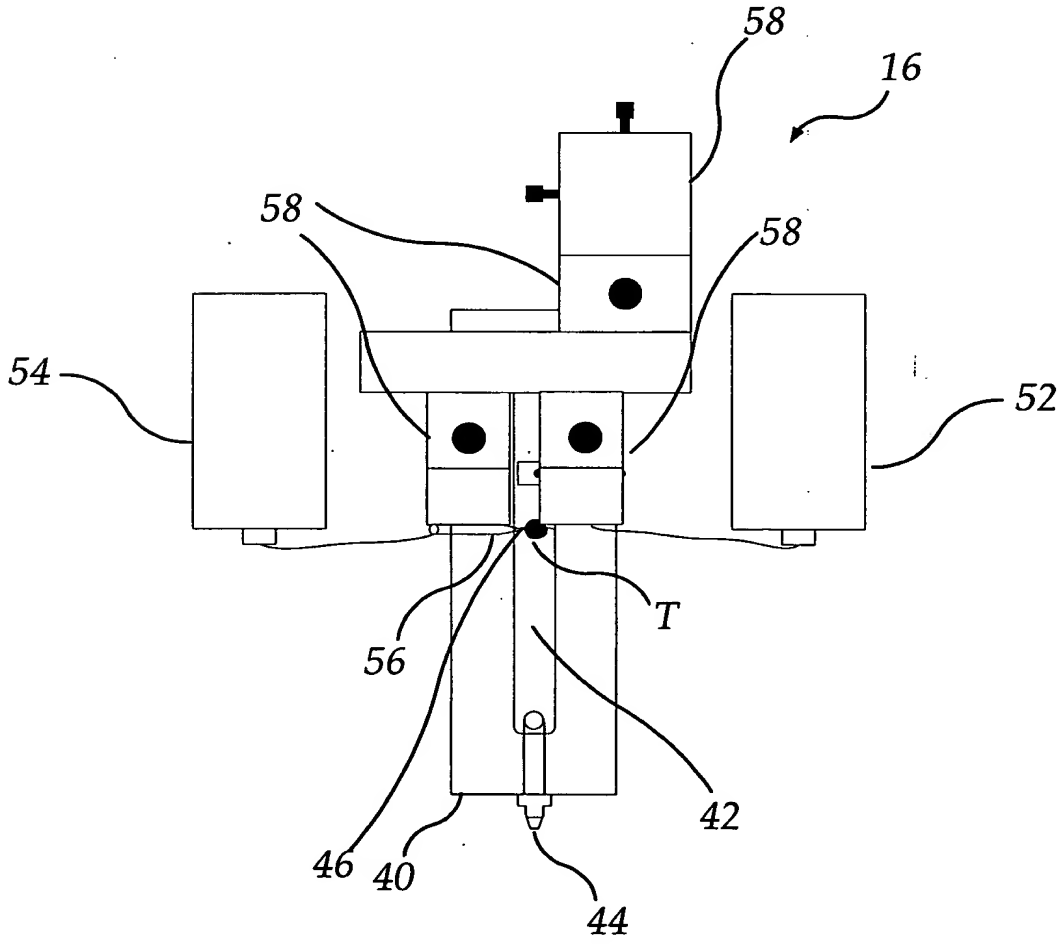


FIG. 3

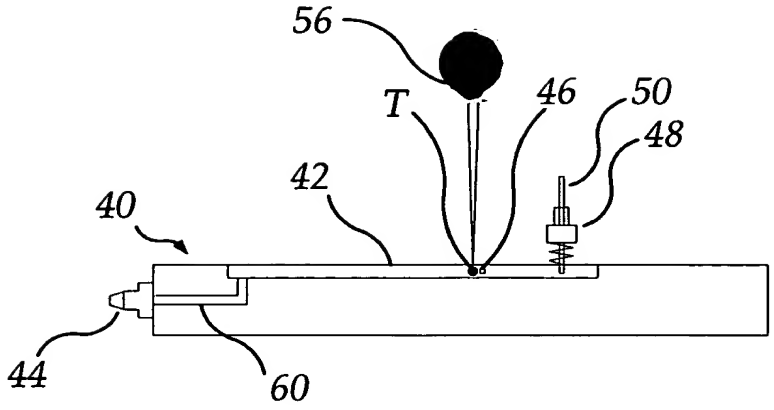


FIG. 4A

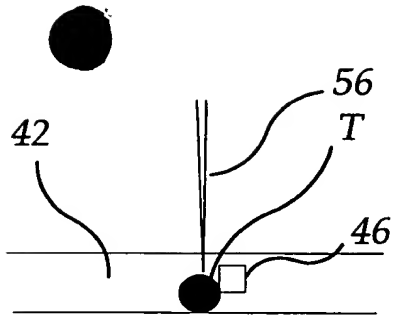


FIG. 4B

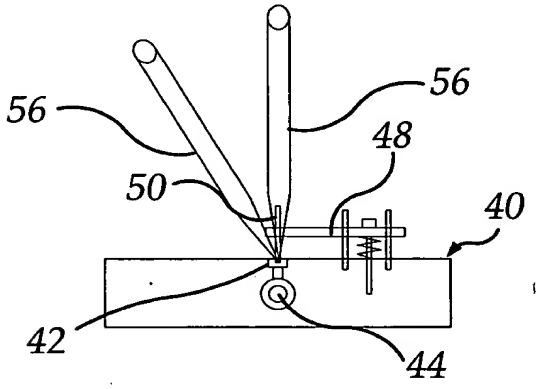


FIG. 4C

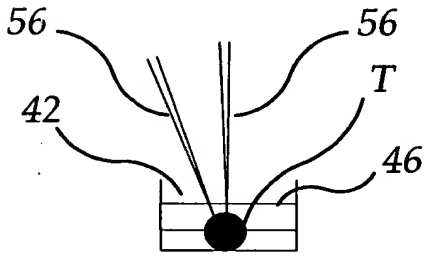


FIG. 4D

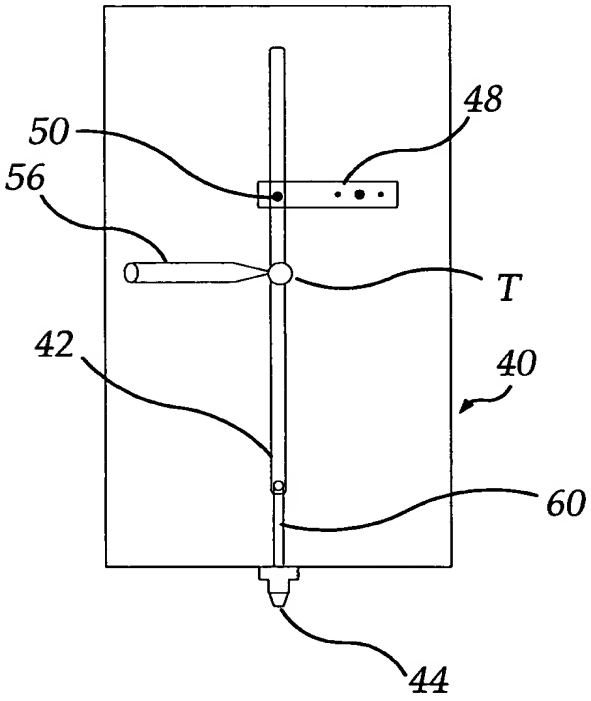


FIG. 4E

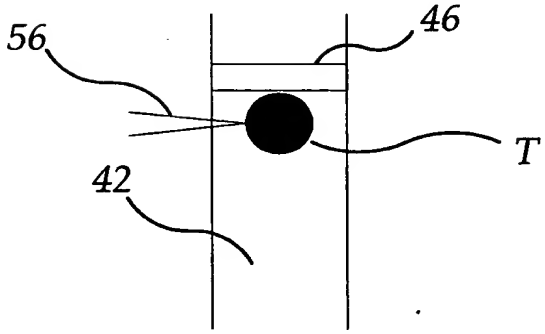


FIG. 4F

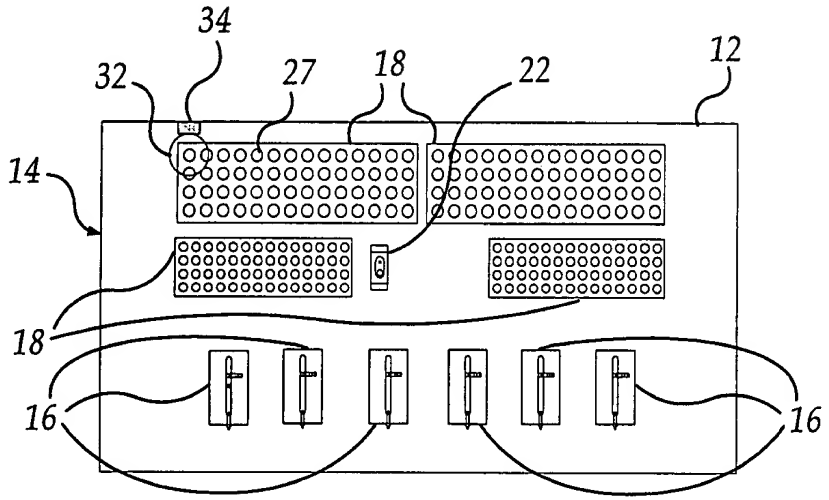


FIG. 5A

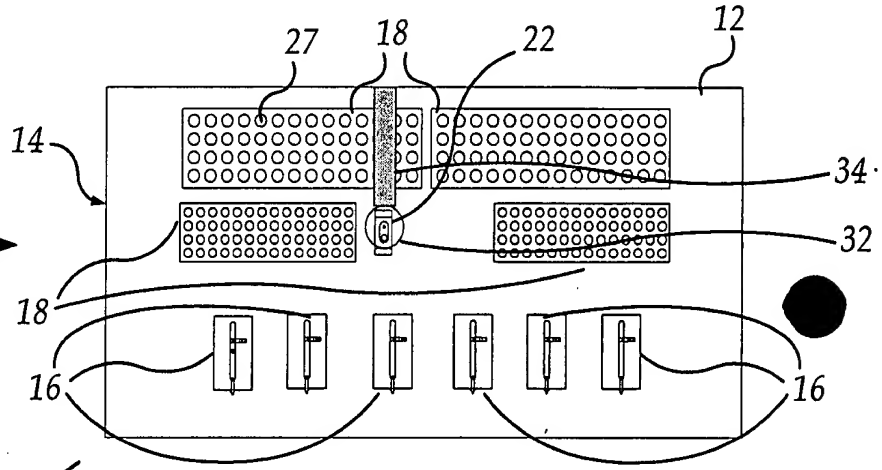


FIG. 5B

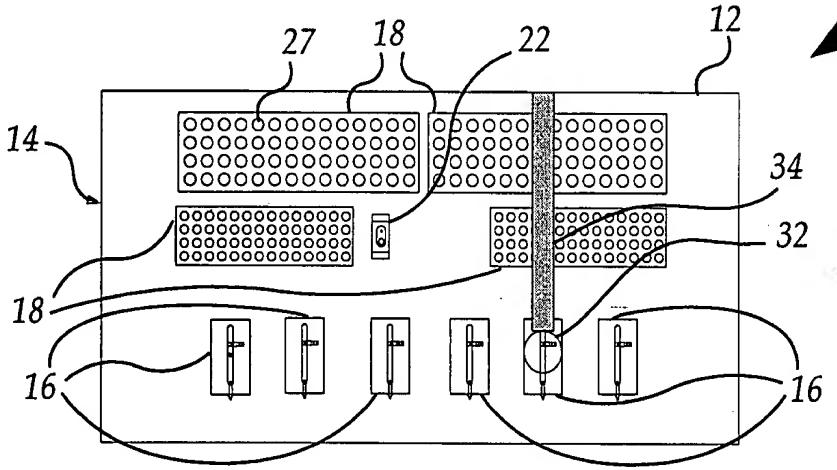


FIG. 5C

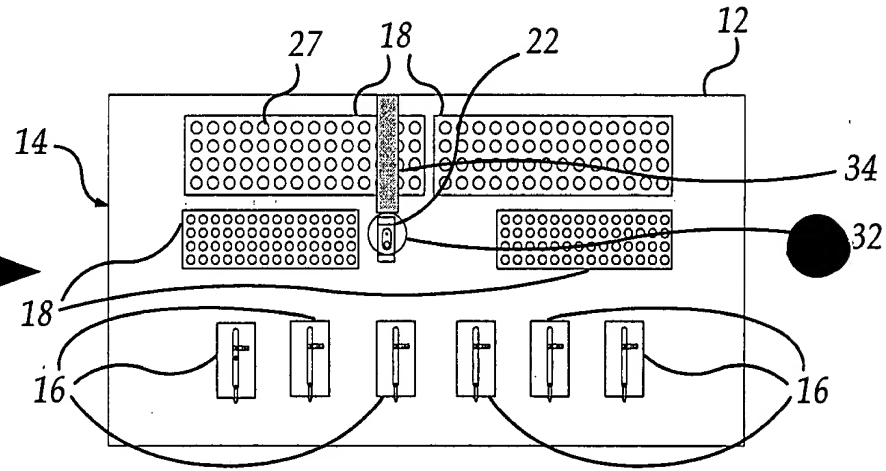
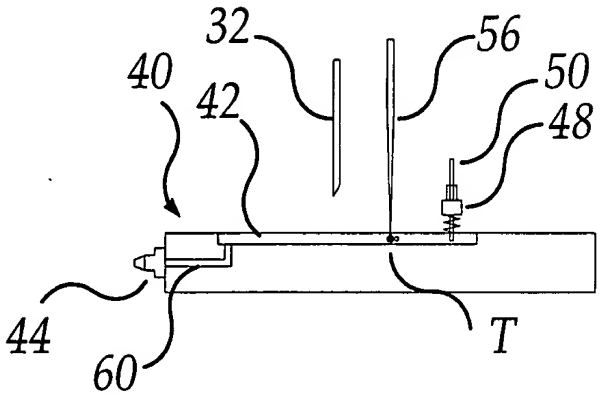


FIG. 5D



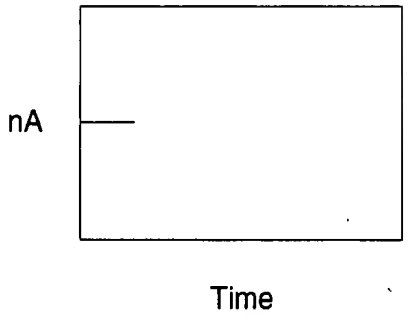
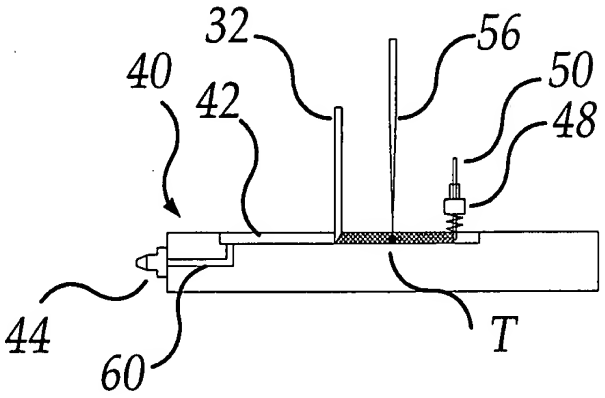


FIG. 6A



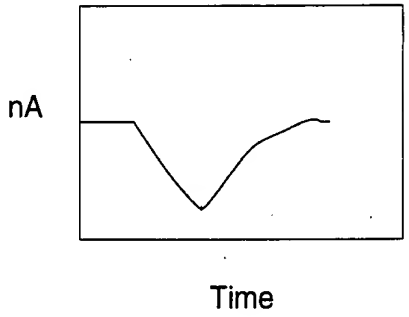
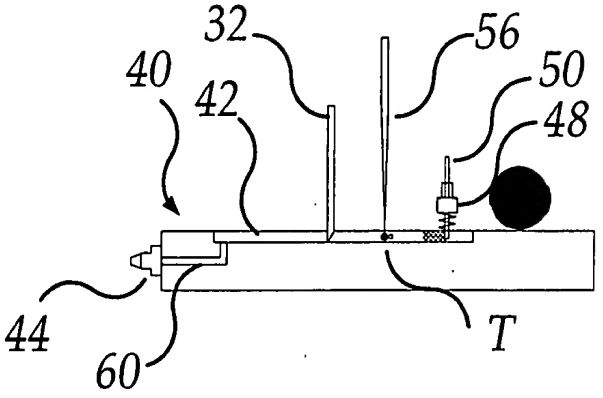


FIG. 6B



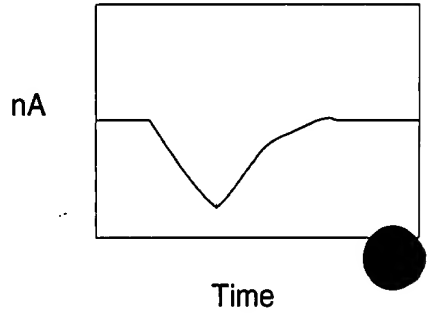


FIG. 6C

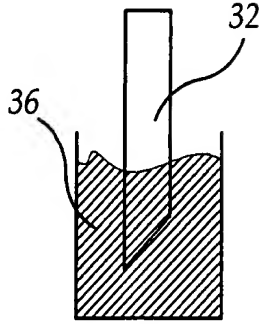


FIG. 7A

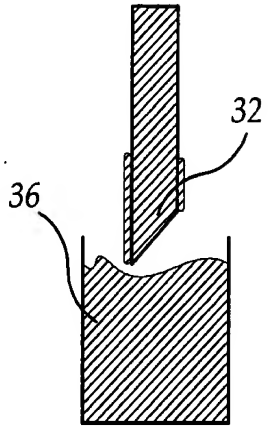


FIG. 7B

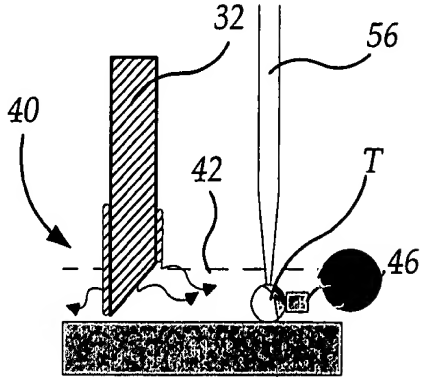


FIG. 7C

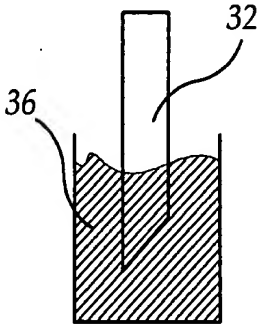


FIG. 7D

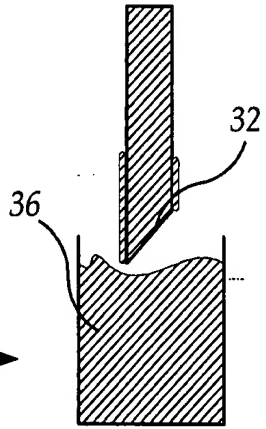


FIG. 7E

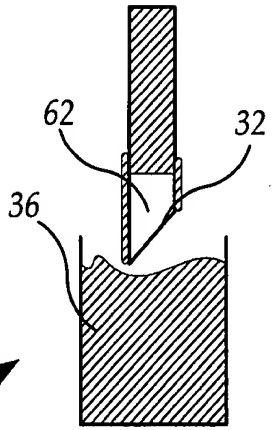


FIG. 7F

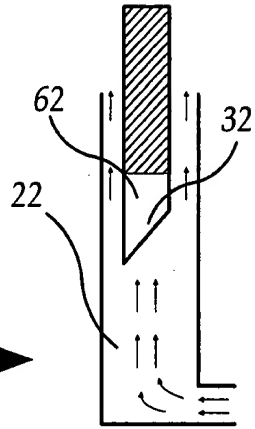


FIG. 7G

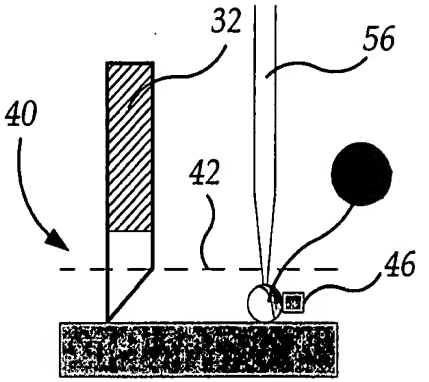


FIG. 7H

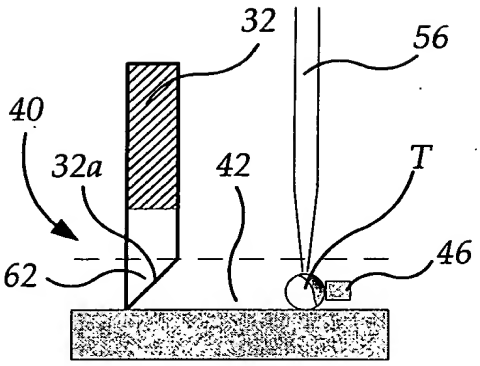


FIG. 8A

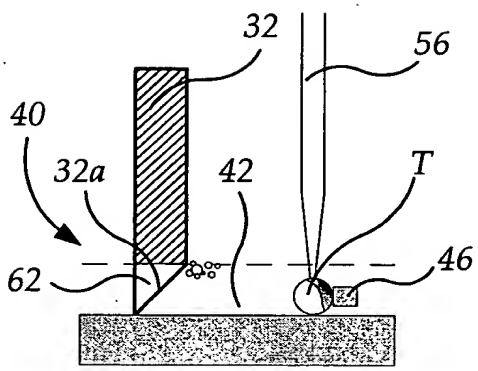


FIG. 8B

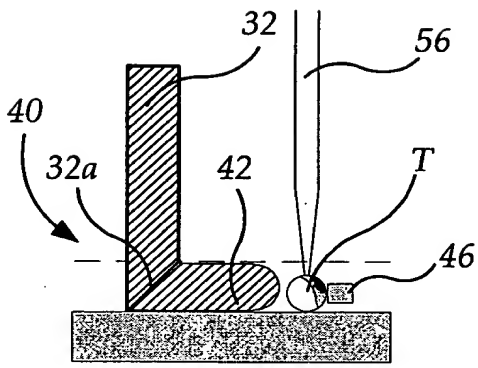


FIG. 8C

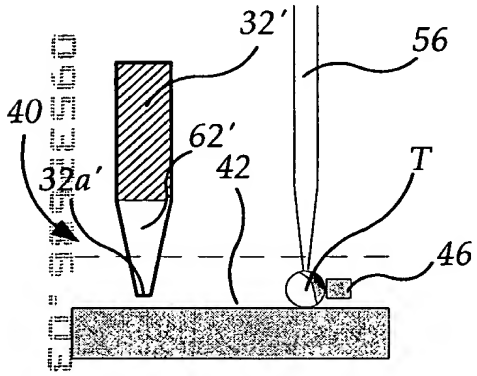


FIG. 8D

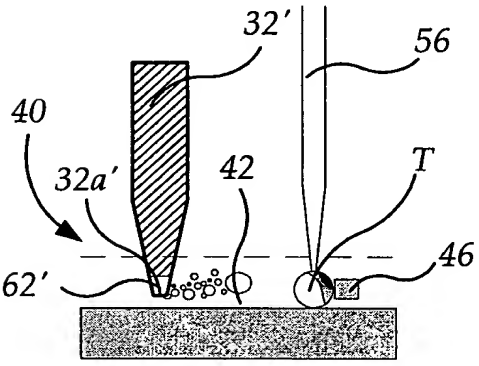


FIG. 8E

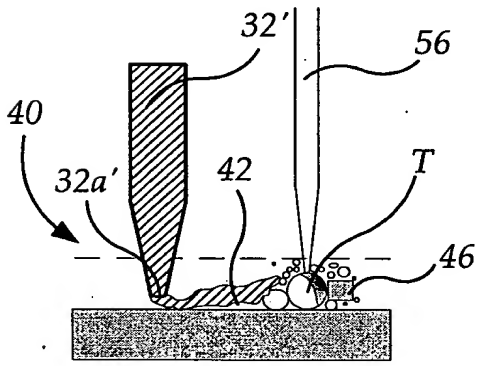


FIG. 8F

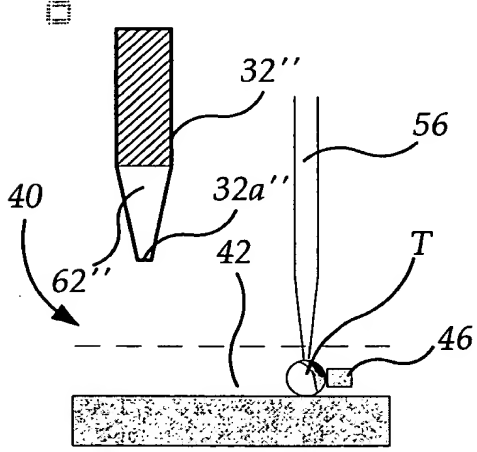


FIG. 8G

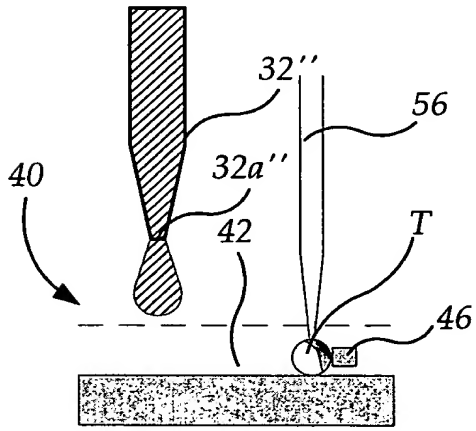


FIG. 8H

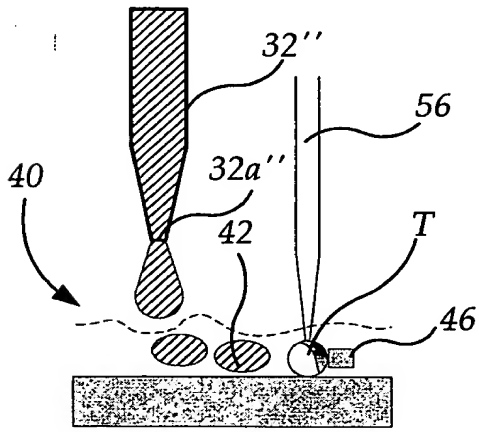


FIG. 8I

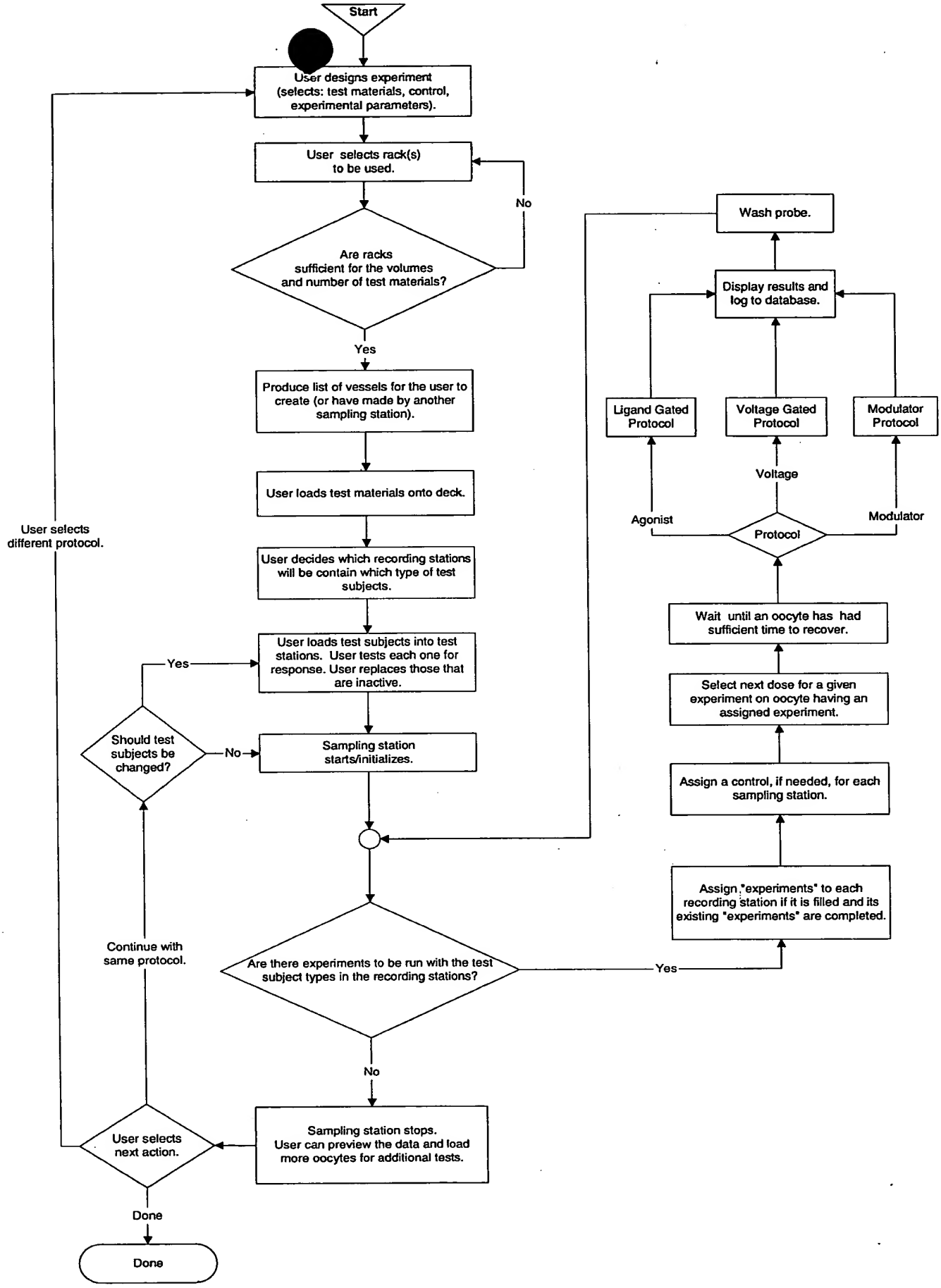

Start
User designs experiment (selects: test materials, control, experimental parameters).
User selects rack(s) to be used.
No
Are racks sufficient for the volumes and number of test materials?
Yes
Produce list of vessels for the user to create (or have made by another sampling station).
User loads test materials onto deck.
User decides which recording stations will be contain which type of test subjects.
User loads test subjects into test stations. User tests each one for response. User replaces those that are inactive.
Yes
Should test subjects be changed?
No
Sampling station starts/initializes.
User selects different protocol.
Continue with same protocol.
Are there experiments to be run with the test subject types in the recording stations?
Yes
No
Sampling station stops. User can preview the data and load more oocytes for additional tests.
User selects next action.
Done
Done
Assign "experiments" to each recording station if it is filled and its existing "experiments" are completed.
Assign a control, if needed, for each sampling station.
Select next dose for a given experiment on oocyte having an assigned experiment.
Wait until an oocyte has had sufficient time to recover.
Protocol
Agonist
Voltage
Modulator
Ligand Gated Protocol
Voltage Gated Protocol
Modulator Protocol
Display results and log to database.
Wash probe.

FIG. 9

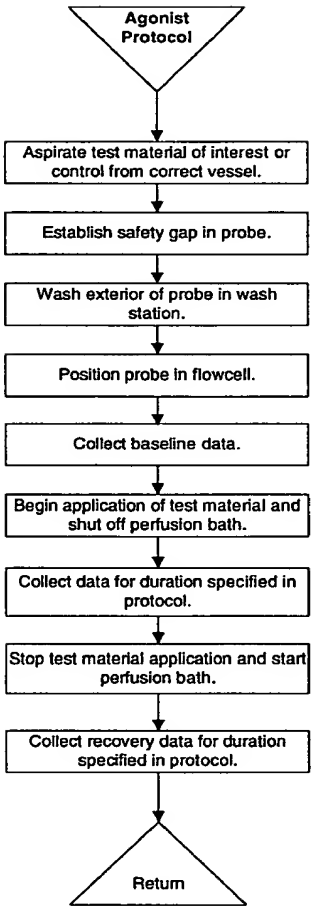

Agonist Protocol
Aspirate test material of interest or control from correct vessel.
Establish safety gap in probe.
Wash exterior of probe in wash station.
Position probe in flowcell.
Collect baseline data.
Begin application of test material and shut off perfusion bath.
Collect data for duration specified in protocol.
Stop test material application and start perfusion bath.
Collect recovery data for duration specified in protocol.
Return

FIG. 10

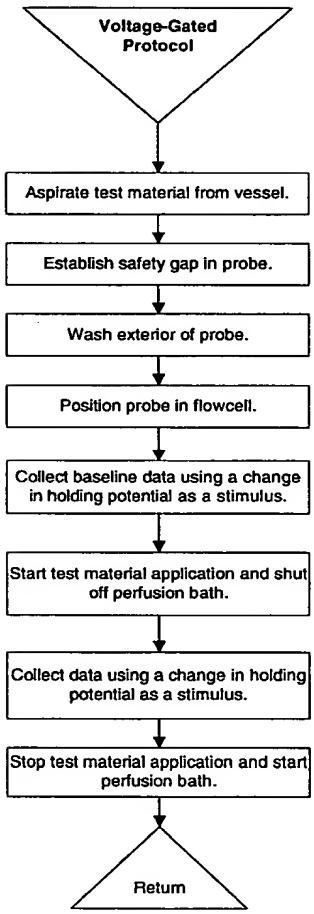

Voltage-Gated Protocol
Aspirate test material from vessel.
Establish safety gap in probe.
Wash exterior of probe.
Position probe in flowcell.
Collect baseline data using a change in holding potential as a stimulus.
Start test material application and shut off perfusion bath.
Collect data using a change in holding potential as a stimulus.
Stop test material application and start perfusion bath.
Return

FIG. 11

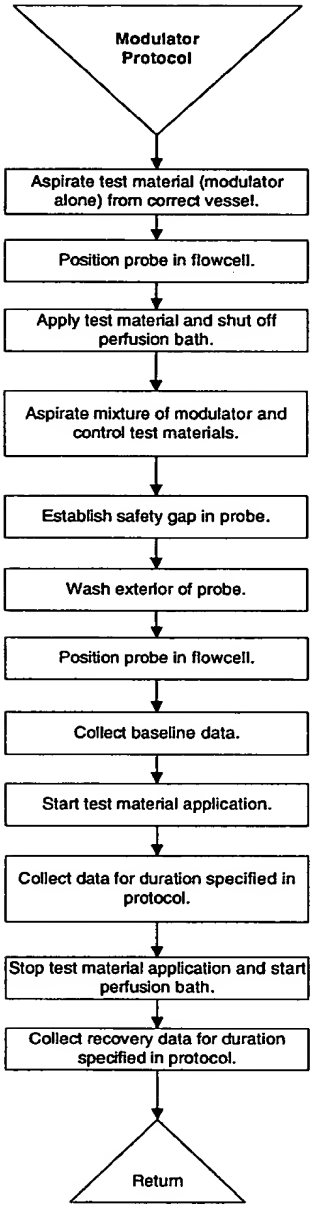

Modulator Protocol
Aspirate test material (modulator alone) from correct vessel.
Position probe in flowcell.
Apply test material and shut off perfusion bath.
Aspirate mixture of modulator and control test materials.
Establish safety gap in probe.
Wash exterior of probe.
Position probe in flowcell.
Collect baseline data.
Start test material application.
Collect data for duration specified in protocol.
Stop test material application and start perfusion bath.
Collect recovery data for duration specified in protocol.
Return

FIG. 12

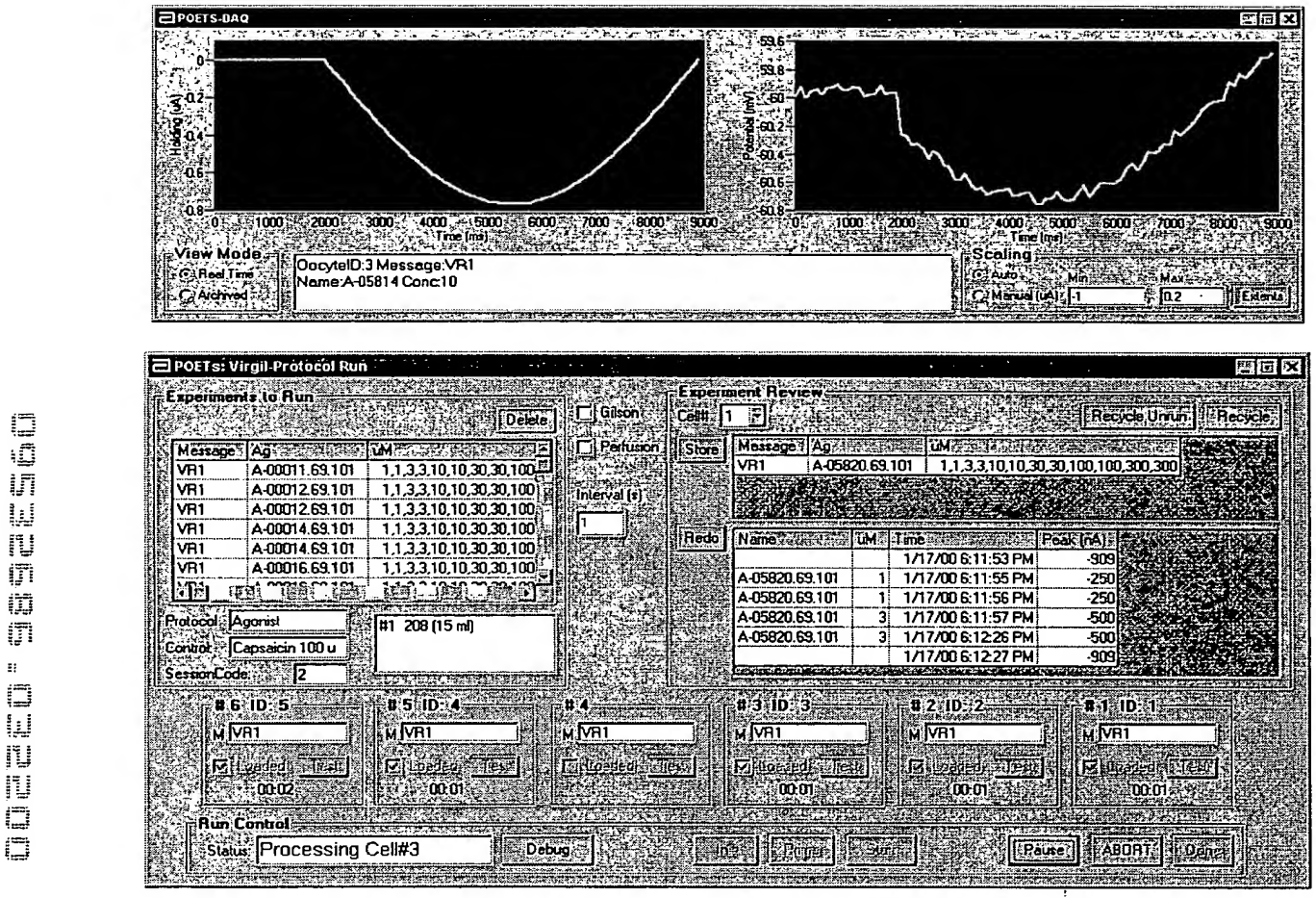

POETS-DAQ
Current (nA)
Potential (mV)
Time (ms)
View Mode
Real Time
Archived
OocyteID:3 Message:VR1
Name:A-05814 Conc:10
Scaling
Auto
Manual (uA)
Min
Max
Extents
POETs: Virgil-Protocol Run
Experiments to Run
Delete
Gilson
Perfusion
Interval (s)
Experiment Review
Cell#
Recycle Unrun
Recycle
Store
Redo
Message
Ag
uM
Name
Time
Peak (nA)
VR1
A-05820.69.101
1,1,3,3,10,10,30,30,100,100,300,300
1/17/00 6:11:53 PM
1/17/00 6:11:55 PM
1/17/00 6:11:56 PM
1/17/00 6:11:57 PM
1/17/00 6:12:26 PM
1/17/00 6:12:27 PM
Protocol
Agonist
Control
Capsaicin 100 u
SessionCode
#1 208 (15 ml)
#6 ID: 5
#5 ID: 4
#4
#3 ID: 3
#2 ID: 2
#1 ID: 1
Loaded
Test
Run Control
Status:
Processing Cell#3
Debug
Pause
ABORT
Done

FIG. 13

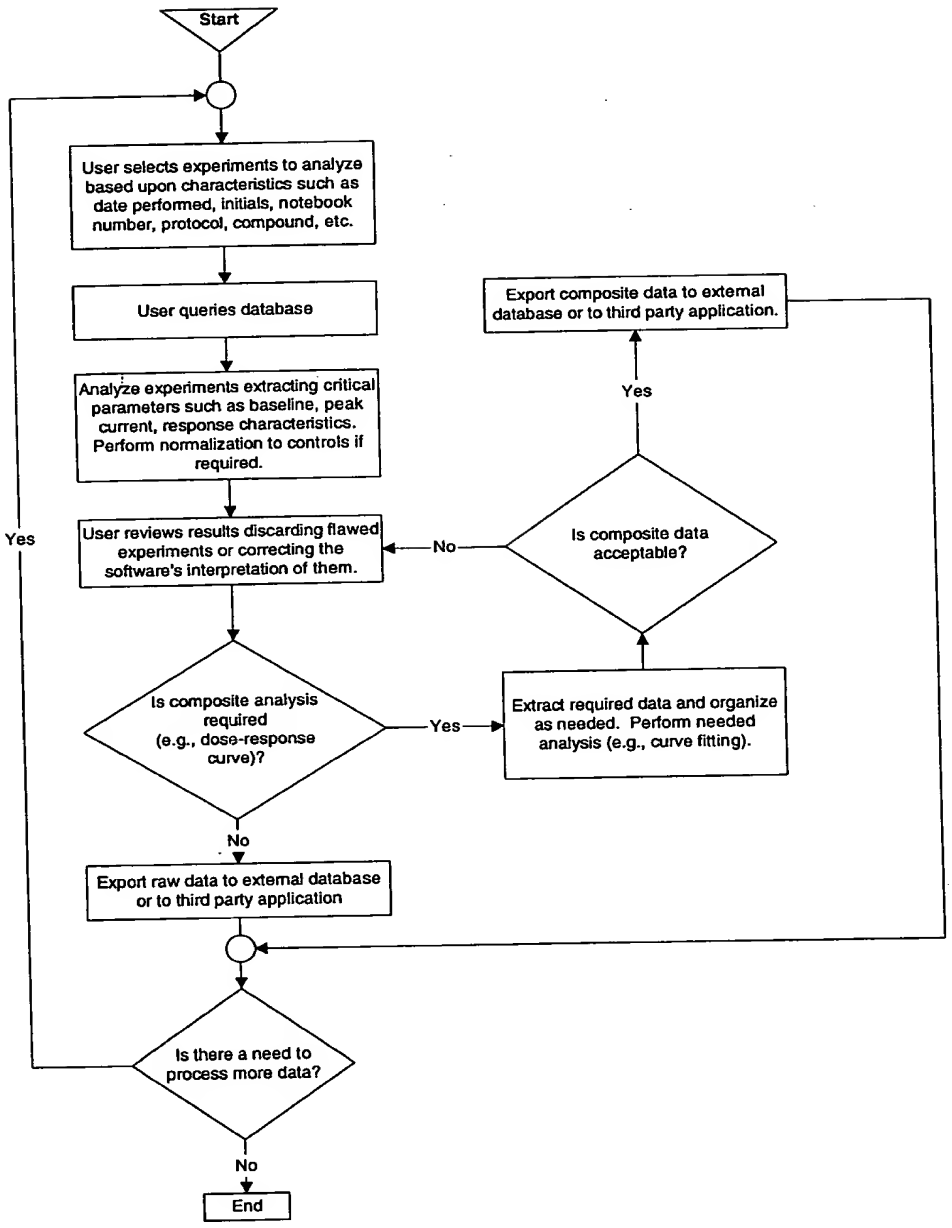

Start
User selects experiments to analyze based upon characteristics such as date performed, initials, notebook number, protocol, compound, etc.
User queries database
Analyze experiments extracting critical parameters such as baseline, peak current, response characteristics. Perform normalization to controls if required.
User reviews results discarding flawed experiments or correcting the software's interpretation of them.
Yes
No
Is composite data acceptable?
Yes
Export composite data to external database or to third party application.
Is composite analysis required (e.g., dose-response curve)?
Yes
Extract required data and organize as needed. Perform needed analysis (e.g., curve fitting).
No
Export raw data to external database or to third party application
Is there a need to process more data?
Yes
No
End

FIG. 14

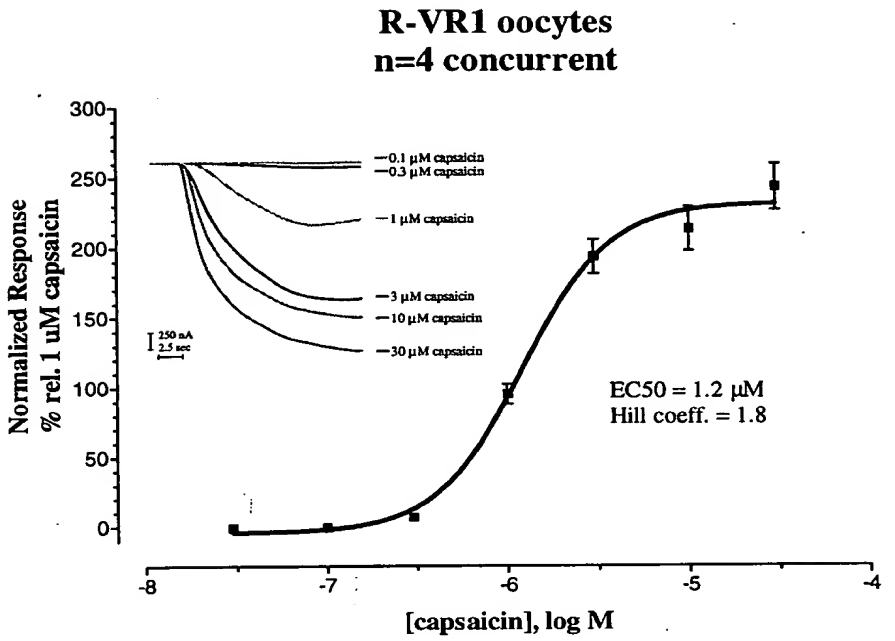

R-VR1 oocytes
n=4 concurrent
Normalized Response
% rel. 1 uM capsaicin
300
250
200
150
100
50
0
0.1 µM capsaicin
0.3 µM capsaicin
1 µM capsaicin
3 µM capsaicin
10 µM capsaicin
30 µM capsaicin
250 nA
2.5 sec
EC50 = 1.2 µM
Hill coeff. = 1.8
-8
-7
-6
-5
-4
[capsaicin], log M

FIG. 15

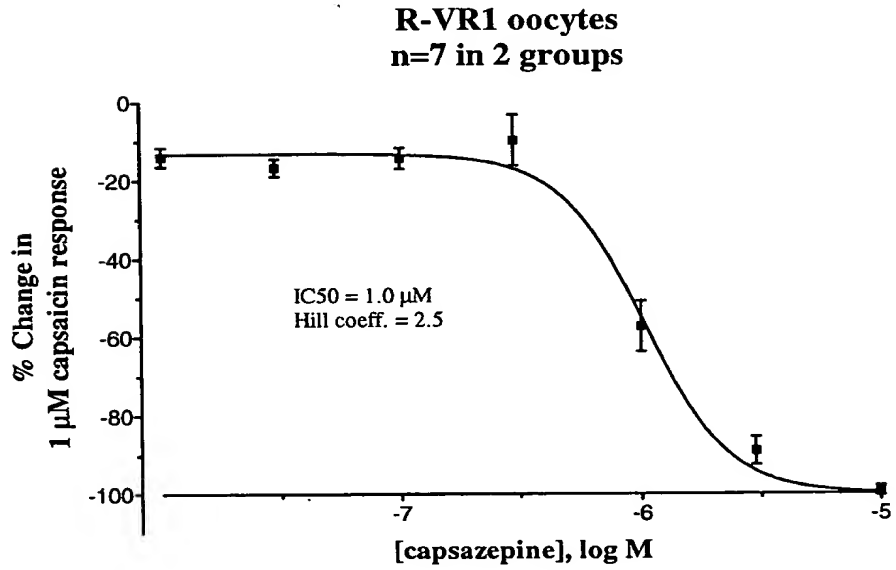

R-VR1 oocytes
n=7 in 2 groups
% Change in
1 µM capsaicin response
0
-20
-40
-60
-80
-100
IC50 = 1.0 µM
Hill coeff. = 2.5
-7
-6
-5
[capsazepine], log M

FIG. 16

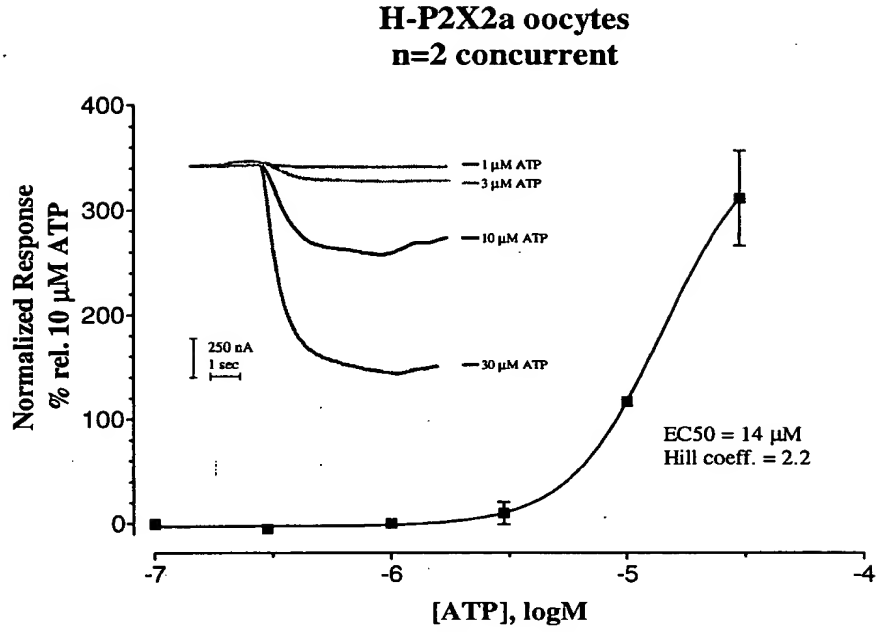

H-P2X2a oocytes
n=2 concurrent
Normalized Response
% rel. 10 µM ATP
400
300
200
100
0
1 µM ATP
3 µM ATP
10 µM ATP
30 µM ATP
250 nA
1 sec
EC50 = 14 µM
Hill coeff. = 2.2
-7
-6
-5
-4
[ATP], logM

FIG. 17

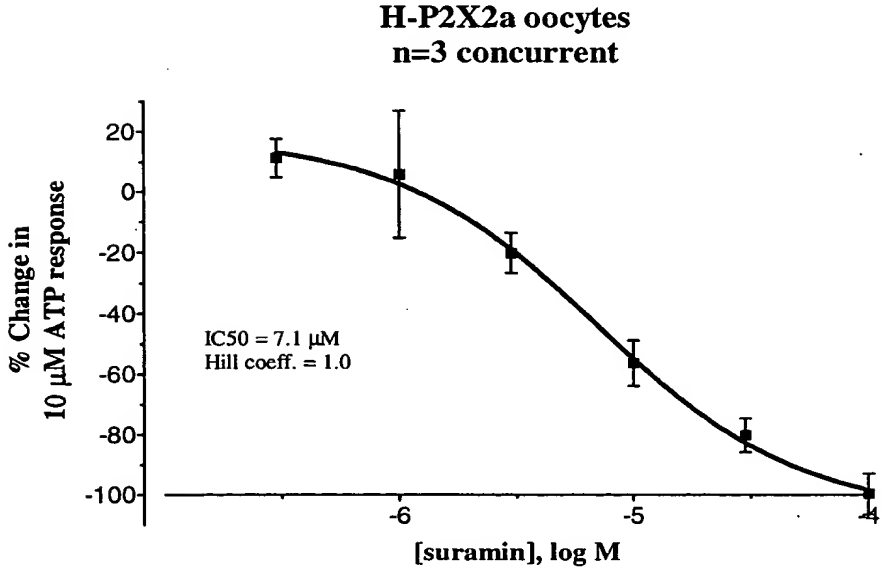

H-P2X2a oocytes
n=3 concurrent
% Change in
10 µM ATP response
20
0
-20
-40
-60
-80
-100
IC50 = 7.1 µM
Hill coeff. = 1.0
-6
-5
-4
[suramin], log M

FIG. 18